Potential of Heated Controlled Atmosphere Postharvest Treatments for the Control of *Thaumatotibia Leucotreta* (Lepidoptera: Tortricidae)

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ABSTRACT Controlled atmosphere/temperature treatment system (CATTS) is an environmentally friendly postharvest mitigation treatment that uses high temperature forced-air combined with a low oxygen and high carbon dioxide atmosphere to control quarantine pests. The development of CATTS treatments is expensive and time-consuming. For a more rapid assessment of different species and life stages' tolerances to heated controlled atmospheres, the controlled atmosphere water bath (CAWB) system can be used to help advance the development of CATTS treatments for pests. The CAWB system was used to test the response of eggs and larval stages of *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae). Eggs and larvae at different developmental stages were treated under regular air and a modified controlled atmosphere of 1% O_2 and 15% CO_2, at two ramping heat rates: 12 and 24°C/h. Typically the faster heat rate and modified atmosphere reduced treatment times required to control the different life stages. *T. leucotreta* larvae were more tolerant of the treatments than eggs. The most tolerant life stage was the fourth instar. Effective treatments against the most tolerant life stage determined by the CAWB system can now be used to develop CATTS technology against *T. leucotreta*. Further research will focus on developing CATTS treatments using infested fruit to determine effective treatments that maintain fruit quality.

KEY WORDS false codling moth, postharvest, heat, controlled atmosphere, CATTS

International trade in agricultural products requires the application of postharvest disinfestation treatments to manage the risk of introducing economically important pests associated with these products, into areas where they do not occur. Fumigation with methyl bromide, a broad spectrum fast-acting fumigant, has been widely used for this purpose since the 1930s (Bell et al. 1996). According to the Montreal Protocol on Substances that Deplete the Ozone Layer, methyl bromide was listed as such a substance in 1992, and a global phase out plan in the production and use of methyl bromide was implemented (Fields and White 2002). Developed countries were to completely phase out their use of methyl bromide by 2005, and developing countries have until 2015 to do so. The use of methyl bromide for quarantine purposes however, is currently exempt from the phase out plan, but this is only until suitable alternative postharvest treatments are developed. The global phase out in the production of methyl bromide will also lead to this treatment becoming more expensive. Some alternatives being investigated include treatments using extreme temperatures, modified controlled atmospheres and irradiation (Heather and Hallman 2008). Such physical treatments are favored over the use of other toxic fumigants or chemical pesticides for environmental, health and economic reasons.

Substantial success is being achieved in developing alternative postharvest disinfestation treatments (Mitcham 2005), but the balance between achieving insect mortality and maintaining fruit quality is a constant concern. Combining treatments improves the efficacy by reducing the time required for insect mortality to be achieved and potentially, reduced treatment times can help to maintain fruit quality. Combining the physical treatments of heat and a modified controlled atmosphere (CA) has led to the development of the controlled atmosphere temperature treatment system (CATTS) (Neven and Mitcham 1996). In this system, the low-oxygen high-carbon dioxide environment inhibits the insect's normal physiological regulatory mechanisms and ultimately impairs their control of respiration, as well as their ability to deal with the added thermal stress of the heated environment. Treatments already developed using this heated CA system include ones to control codling moth, *Cydia pomonella* L., and western cherry fruit fly, *Rhagoletis indifferens* Curran, in sweet cherries (*Prunus* spp.), and codling moth and oriental fruit moth, *Grapholita molesta* Busck in apples (*Malus* spp.) and peaches and nectarines (both *Prunus* spp.) (Neven 2005; Neven and Rehfield-Ray 2006a,b; Neven et al. 2006). These treatments also have been entered in the USDA–APHIS Quarantine Treatment Manual (2008) and have been demonstrated to be attainable in 2-ton

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commercial-scale chambers (Neven and Rehfield-Ray 2006b). Researchers have shown that CATTS treatments for pome and stone fruit do not compromise commodity market quality, and in some instances improve certain quality parameters (Neven et al. 2001, Obenland et al. 2005). Testing and developing CATTS quarantine treatments is time consuming and expensive due to all the parameters that must be accurately controlled. As for most any treatment, the most tolerant species and life stage must be assessed, and for internal pests treatments must be carried out on infested fruit samples. To determine the most tolerant pest species and life stage to CATTS, without the expense of in-fruit treatments, simulated-CATTS treatments using a water bath system have been developed previously (Neven 2008). This controlled atmosphere water bath (CAWB) system allows for more rapid assessment of target pest species and gives a good indication of the insect's thermotolerance and response to CATTS.

The CAWB system was used to test combined heat and modified controlled atmosphere treatments on the eggs and larvae of *Thaumatotibia leucotreta* (Meyrick) (Lepidoptera: Tortricidae), to assess the potential of developing CATTS treatments for this pest in the future. *T.* commonly known as "false codling moth" in Africa, is a key phytosanitary pest of South African export fruit, because it affects a variety of fruit types, is indigenous to South Africa and has very limited global distribution (De Villiers et al. 1987, van den Berg 2001, CABI 2007). The larvae burrow into fruit to feed, posing a quarantine risk as an internal fruit pest. *T. leucotreta* was first reported as a citrus pest in 1901 but has now been recorded on a wide variety of cultivated and wild host plants (van den Berg 2001, Kirkman and Moore 2007). Export fruit from South Africa most affected by *T. leucotreta* are citrus and stone fruits. Studies of citrus fruit tolerances to high-temperature forced air treatments, such as those used in CATTS, have indicated high tolerance levels and good potential for the development of CATTS treatments for citrus (Shellie et al. 1993, Sharp and McGuire 1996, Shellie and Mangan 1998). The tolerance of peach and nectarine cultivars to CATTS treatments was established by Obenland et al. (2005). Results also indicated a high tolerance to such heat treatments and no negative effects on fruit marketability. Inferring the results from such studies, together with that from the current study of pest tolerance, will help establish relative tolerance of *T. leucotreta* to CATTS treatments, and speed up the development thereof.

Management strategies for preharvest control of *T. leucotreta* reduce damage in orchards, and cultural practices for postharvest control reduce the threat of infestation, but specific postharvest treatments are limited. Other than cold storage treatment, which is not feasible for all fruit types, there is a lack of effective postharvest alternatives to methyl bromide fumigation to ensure quarantine security against *T. leucotreta* for countries importing fruit from South Africa.

Materials and Methods

Insects. A laboratory colony of *T. leucotreta* was established in 2007, maintained on an artificial diet (Guennelon et al. 1981), and reared at ±26°C, ±65% RH, and a photoperiod of 18:6 (L:D) h to provide eggs and larvae for treatments. Adult moths were placed under a sieve on wax paper and allowed to oviposit for 24–48 h under the rearing conditions described above. Wax paper with eggs was removed from under the sieve and left for eggs to develop to the different egg stages before treatment. White eggs developed into red eggs within 3–4 d and finally into black head eggs within 5–6 d. To obtain larvae, wax paper sheets with eggs were sterilized in a 1% Sporekill (ICA Laboratories, Cape Town, South Africa) solution for four minutes, followed by a 2% sodium hypochlorite solution for 4 min and then rinsed in water for 4 min. Egg sheets were placed on the artificial diet and held under the rearing conditions described above to allow for larval development to different instars for use in testing treatments.

CAWB System. A controlled atmosphere water bath system and insect container, based on that described by Neven (2008), was used to test treatments (Fig. 1). The system is comprised of a programmable water bath and an O_2/CO_2 gas analyzer. The water bath used was a Grant R4 tank (20-liter capacity) and GP200 thermostatic controller (Grant Instruments Ltd., Cambridgeshire, England) connected to a computer. Grant Labwise software was used to setup and control temperature programs. The O_2/CO_2 gas analyzer was a PDA 400 (Pacific CA Systems, Yakima, WA), with gas concentration ranges of 0–21% O_2 and 0–20% CO_2. The flow rate was monitored by a flow meter attached to the gas analyzer (0–1 liters/min). Premixed cylinders of gas containing 1% O_2, 15% CO_2 and balance N_2 were used to supply the modified CA to the insect containers. Gas was released from the cylinder by a two-stage regulator and pumped through the gas analyzer, to monitor gas concentrations and flow rate, and then into the insect container. Treatments were also run with regular room air (RA), to determine the effect of heat alone, in which case room air was pumped by the gas analyzer into the insect container. The flow rate of gas through the system was 1 liter/min. The insect container was a rectangular clear Perspex box (340 by 240 by 110 mm) with a rubber-lined lid that was clamped down, and gas inlet and outlet attachments. Glass test tubes (10 by 16 mm) extended from the bottom of the Perspex box and housed the insects, which were confined to the bottom quarter of the test tube by modified plastic Pasteur pipettes and organza cloth. There were 48 test tubes secured into the Perspex box. The bottom of the box rested on the edges of the opening of the water bath tank with the test tubes extending from the bottom of the box into the water. Water-soaked sponges glued to the inside of the box were used to maintain humidity in the sealed insect container during treatments. Humidity in the insect container was monitored using iButton data loggers and Climastats software (Fair-

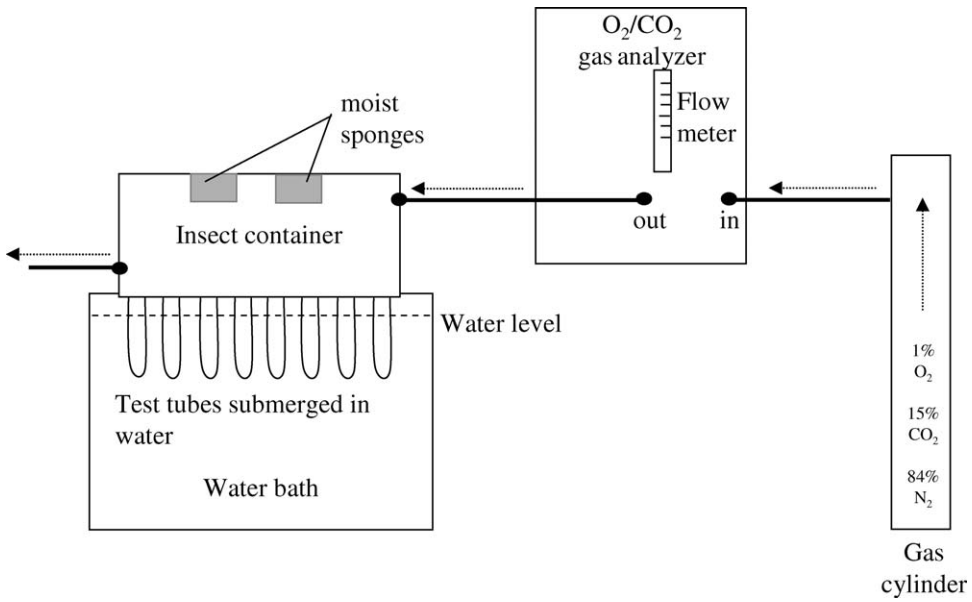

Fig. 1. Diagram of the CAWB system. For modified controlled atmosphere treatments the system consists of a gas cylinder with a commercial mixture of 1% O_2, 15% CO_2, and balance N_2. Premixed air is pumped via the gas analyzer into the insect container. For regular air treatments the gas cylinder was detached and room air was pumped through the system. The solid black lines represent the air pipeline. Arrows represent the direction of ga flow. For a detailed description of the system see text.

Bridge Technologies, Gauteng, South Africa). Temperatures inside the test tubes were monitored using thermocouples and a KM22 digital thermometer (Kany-May, Herfordshire, England).

Treatments. The start temperature of water for all treatments was 23°C and was increased to 45°C at one of two heating rates programmed into the water bath, a slower rate of 12°C/h and a faster rate of 24°C/h. Both heating rates were applied with the modified CA and with RA. For treatment of eggs, wax paper sheets with eggs at each developmental stage, white, red, and black head, were cut into small strips and placed inside the test tubes in the insect container. The number of eggs tested per replicate ranged from 250 to 350 eggs. For treatments of *T. leucotreta* larvae (first–fifth instars), 20 insects were used per replicate. As control treatments, equal numbers of eggs and larvae were placed into test tubes and left on the bench for the duration of each trial. Before starting treatments, the open insect container with eggs or larvae in test tubes was lowered into the water bath and left for 15 min to allow the larvae to settle and for the temperature to stabilize at 23°C. When treatments were started the lid of the container was clamped down, heat ramping initiated and gas flow started. Heat ramping to a final temperature of 45°C at a linear rate of 12°C/h took 110 min, and 55 min at a heat ramping rate of 24°C/h. Once reached, the final temperature was maintained for the duration of treatment. The effect of each treatment was assessed at 30-min incremental time points after the start of treatment. At each time point gas flow was stopped and the insect container removed. Each time point per treatment was replicated four times. After treatment larvae and egg sheets were removed from test tubes to assess mortality. First–fourth instars were placed in ventilated 500-ml plastic containers on artificial diet and held at ±26°C, ±65% RH, and a photoperiod of 18:6 (L:D) h. Larval survivorship was assessed after 7 d, and numbers of live and dead larvae were determined. Live larvae were kept under these conditions to continue development until moth emergence. Fifth instars were placed in petri dishes and covered with fine sawdust to facilitate pupation and also held at ±26°C, ±65% RH and a photoperiod of 18:6 (L:D) h. Survival was assessed after 14 d when the number of emerged moths was counted. Treated egg sheets were held under the same rearing conditions for 7 d, after which numbers of hatched and unhatched eggs were counted.

Statistics. Treatment mortality for *T. leucotreta* eggs was control corrected using Henderson–Tilton's formula because nonuniform numbers of test and control eggs were used (Henderson and Tilton 1955). Treatment mortality for *T. leucotreta* larvae was corrected for control mortality using Abbott's formula because uniform numbers of test and control insects were used (Abbott 1925). The mean percentage of corrected mortality and standard error was determined across replicates for each time point. Mean percentage of corrected mortalities were arcsine $\sqrt{x}$ transformed for further analysis. Factorial analysis of variance was performed on the transformed data of each test subject in each treatment using STATISTICA version 8 (StatSoft, Tulsa, OK). Significantly different means were separated using Tukey's honest significance difference test.

Table 1. Mean percentage of corrected mortality ± SE of *T. leucotreta* egg stages after RA and modified CA heat treatments at 12°C/h heating rate

Time (h)	White		Red		Black	
	RA	CA	RA	CA	RA	CA
0.5	16.24 ± 2.12a	23.36 ± 1.04x	4.13 ± 1.95b	11.11 ± 0.64x	17.89 ± 3.65a	17.03 ± 3.88x
1.0	35.77 ± 2.03a	29.56 ± 3.69x	7.96 ± 0.89b	14.65 ± 2.23x	16.26 ± 2.70b	11.85 ± 3.03y
1.5	50.06 ± 1.50a	37.83 ± 1.37x	10.58 ± 1.81b	20.35 ± 1.85x	17.65 ± 2.07b	63.35 ± 4.03y
2.0	38.25 ± 5.78a	52.89 ± 4.74x	15.66 ± 1.24b	96.49 ± 0.42y	35.94 ± 2.39a	99.77 ± 0.16y
2.5	58.12 ± 5.23a	100 ± 0.00x	19.28 ± 2.13b	100 ± 0.00x	29.46 ± 3.20b	100 ± 0.00x

CA conditions were 1% O_2 and 15% CO_2. Start temperature was 23°C and end temperature was 45°C. Times are total times from start of the heat treatment. n = 250–350 per treatment.

In each row, for each time point under RA, different letters (a, b) indicate significant differences between means.

In each row, for each time point under CA, different letters (x, y) indicate significant differences between means.

Results

Relative humidity in the insect container during RA treatments averaged 80.0 ± 1.3% and 69.7 ± 3.0% during CA treatments. Temperatures in the test tubes lagged 1–2°C behind the water temperature as the water bath temperature ramped up at both heating rates. Test tube temperatures reached the final temperature (45°C) after 3 h when the ramping heat rate was 12°C/h and after 2 h at the 24°C/h heat rate.

In all treatments of egg developmental stages and instars, at both heating rates and across the time points, the percentage of corrected mortality was higher when the controlled atmosphere was applied than under heat treatment alone ($P < 0.05$). Although in some cases, at early time points (0.5, 1.0, and 1.5 h), mortality under CA was lower than under RA, at effective treatment times, when 100% mortality was reached, the effect of CA as the causal factor was evident.

In the 12°C/h RA treatment on *T. leucotreta* eggs, the different egg stages responded differently to the treatment ($F_{8, 45} = 5.57$; $P < 0.05$) (Table 1). White eggs were less tolerant to heat than red and black head eggs, which were equally thermotolerant to one another at three of the time points used (1.0, 1.5, and 2.5 h). However, white eggs were found to be the most tolerant to the CA treatment ($F_{8, 45} = 49.01$; $P < 0.05$), with half the mortality as that observed in red and black head eggs at the 2.0-h time point. After 2.5 h, however, all three egg developmental stages had reached 100% mortality.

In contrast to the response of egg stages to heat alone at the slower heat rate, when the heat rate was doubled to 24°C/h, no egg stage was more tolerant of the RA treatment than another ($F_{6, 36} = 2.01$; $P = 0.09$) (Table 2). When the modified controlled atmosphere was applied, initially white and red eggs were equally tolerant of the 24°C/h CA treatment, and both were more tolerant than black head eggs ($F_{6, 36} = 6.77$; $P < 0.05$). Later, at 1.5- and 2.0-h time points, no stage was more tolerant than another and effective treatment times were reached.

The time taken for 100% mortality to be achieved in the most tolerant egg developmental stage at the slower heat rate was 2.5 h. The faster heat rate reduced effective treatment time against the most tolerant egg stage to 2.0 h.

In the 12°C/h RA treatment of *T. leucotreta* larvae, the different instars responded differently to the treatment of heat alone ($F_{16, 75} = 2.72$; $P < 0.05$) (Table 3). At the later 2.0- and 2.5-h time points, first and second instars were equally thermotolerant to one another, and less thermotolerant than the third–fifth instars (which were also equally thermotolerant to one another). When the modified controlled atmosphere was applied, at the effective treatment time (2.5 h), the first and fifth instars were equally tolerant of the treatment but less so than second and third instars. The fourth instar was observed to be the most tolerant life stage ($F_{16, 75} = 6.89$; $P < 0.05$) under these conditions.

The response of larvae to the RA treatment at the 24°C/h heat ramp rate was also different between different instars ($F_{16, 75} = 1.99$; $P < 0.05$) (Table 4) and showed the same levels of thermotolerance as at 12°C/h: at 2.0- and 2.5-h time points, first and second

Table 2. Mean percentage corrected mortality ± SE of *T. leucotreta egg* stages after RA and modified CA heat treatments at 24°C/h heating rate

Time (h)	White		Red		Black	
	RA	CA	RA	CA	RA	CA
0.5	6.73 ± 2.02a	2.17 ± 1.75x	8.65 ± 5.98a	3.56 ± 2.33x	9.36 ± 0.66a	10.45 ± 0.86y
1.0	14.43 ± 2.53a	5.68 ± 1.73x	4.85 ± 1.58a	7.99 + 2.07x	12.01 ± 2.24a	22.30 ± 1.04y
1.5	13.07 ± 3.82a	99.59 ± 0.35x	10.00 ± 0.85a	99.69 ± 0.31x	4.74 ± 0.62a	100 ± 0.00x
2.0	12.10 ± 1.96a	100 ± 0.00x	11.54 ± 2.36a	100 ± 0.00x	9.49 ± 2.49a	100 ± 0.00x

CA conditions were 1% O_2 and 15% CO_2. Start temperature was 23°C and end temperature was 45°C. Times are total times from start of the heat treatment. n = 250–350 per treatment.

In each row, for each time point under RA, different letters (a, b) indicate significant differences between means.

In each row, for each time point under CA, different letters (x, y) indicate significant differences between means.

Table 3. Mean percentage corrected mortality ± SE of *T. leucotreta* larvae after RA and modified CA heat treatments at 12°C/h heating rate

Time (h)	First		Second		Third		Fourth		Fifth	
	RA	CA	RA	CA	RA	CA	RA	CA	RA	CA
0.5	20.00 ± 6.12a	23.75 ± 2.39x	10.00 ± 2.04a	31.25 ± 4.27x	5.00 ± 2.04a	12.50 ± 3.23x	8.75 ± 2.39a	20.00 ± 2.06x	3.75 ± 1.25a	16.25 ± 2.39x
1.0	33.75 ± 6.88a	27.50 ± 5.02x	12.50 ± 4.79a	32.50 ± 6.61x	10.00 ± 2.04b	13.75 ± 3.15x	8.75 ± 3.15b	21.25 ± 2.39x	17.50 ± 5.95a	33.75 ± 7.47x
1.5	35.00 ± 3.54a	43.75 ± 5.54x	20.00 ± 2.05a	37.50 ± 3.23x	20.00 ± 3.54a	18.75 ± 3.35x	27.5 ± 5.95a	26.25 ± 4.27x	16.25 ± 4.27a	32.50 ± 7.77x
2.0	52.50 ± 4.33a	53.75 ± 1.25x	40.00 ± 2.04a	52.50 ± 7.50x	21.25 ± 3.75b	40.00 ± 11.73x	26.25 ± 2.38b	26.25 ± 3.15y	17.50 ± 3.23b	45.00 ± 12.25x
2.5	50.00 ± 6.12a	100 ± 0.00x	42.50 ± 1.44a	70.00 ± 5.40y	26.25 ± 2.39b	53.75 ± 6.88y	18.75 ± 5.91b	31.25 ± 3.15y	15.00 ± 2.04b	95.00 ± 3.54x

CA conditions were 1% O_2 and 15% CO_2. Start temperature was 23°C and end temperature was 45°C. Times are total times from start of the heat treatment. $n = 80$ for all treatments.
In each row, for each time point under RA, different letters (a, b) indicate significant differences between means.
In each row, for each time point under CA, different letters (x, y) indicate significant differences between means.

Table 4. Mean percentage corrected mortality ± SE of *T. leucotreta* larvae af Tim e ter RA and modified CA heat treatments at 24°C/h heating rate

Time (h)	First		Second		Third		Fourth		Fifth	
	RA	CA	RA	CA	RA	CA	RA	CA	RA	CA
0.5	26.25 ± 3.15a	55.00 ± 4.56x	25.00 ± 8.90a	25.00 ± 8.16y	17.50 ± 3.23a	16.25 ± 3.75y	12.5 ± 5.95a	13.75 ± 3.15y	13.75 ± 3.75a	1.25 ± 1.25z
1.0	27.50 ± 3.23a	52.50 ± 5.20x	37.50 ± 3.23a	37.50 ± 5.20x	20.00 ± 3.54a	15.00 ± 2.04y	8.75 ± 4.27a	12.50 ± 5.20y	23.75 ± 5.54a	5.00 ± 2.89y
1.5	41.25 ± 4.27a	65.00 ± 5.40x	37.50 ± 5.20a	42.50 ± 2.50x	23.75 ± 4.27a	26.25 ± 1.25y	25.00 ± 4.08a	20.00 ± 3.54y	25.00 ± 4.08a	52.50 ± 5.95x
2.0	48.75 ± 4.73a	98.75 ± 1.25x	46.25 ± 3.15a	63.75 ± 13.90y	31.25 ± 1.25b	52.50 ± 6.29y	26.25 ± 2.39b	58.75 ± 3.15y	21.25 ± 8.51b	98.75 ± 1.25x
2.5	78.78 ± 4.27a	100 ± 0.00x	58.75 ± 3.75a	85.00 ± 4.56y	32.5 ± 10.41b	83.75 ± 8.26y	33.75 ± 4.73b	72.50 ± 4.33y	26.25 ± 1.25b	100 ± 0.00x

CA conditions were 1% O_2 and 15% CO_2. Start temperature was 23°C and end temperature was 45°C. Times are total times from start of the heat treatment. $n = 80$ for all treatments.
In each row, for each time point under RA, different letters (a, b) indicate significant differences between means.
In each row, for each time point under CA, different letters (x, y) indicate significant differences between means.

instars were equally thermotolerant to one another and less thermotolerant than the third, fourth, and fifth instars (which were also equally thermotolerant to one another). Similarly, as with the slower heating rate, under the modified controlled atmosphere, at the effective treatment time (2.5 h), second, third, and fourth instars were more tolerant than first and fifth instars, and the fourth instar was the most tolerant of the treatment ($F_{16,\ 75} = 8.44$; $P < 0.05$).

For the duration of treatments tested here, 100% mortality was not reached in the most tolerant instar. Mortalities achieved for the most tolerant fourth instar when treated with a modified controlled atmosphere for 2.5 h were 31.25 ± 3.15 and 72.5 ± 4.33% at the 12 and 24°C/h ramping heat rates, respectively. In addition, *T. leucotreta* larvae are more tolerant of heated CA treatments than the eggs.

Discussion

In previous research using the CAWB system on the eggs of codling moth and oriental fruit moth, the egg stages of each species were found to be equally thermotolerant to one another, as well as equally tolerant of the controlled atmosphere treatment at a heating rate of 24°C/h (Neven 2008). Similarly, the eggs stages of *T. leucotreta* were also equally thermotolerant under RA treatments at 24°C/h; but in contrast to codling moth and oriental fruit moth, under CA the different egg stages of *T. leucotreta* responded differently. Because white and red eggs were more tolerant than black head eggs at a ramping heat rate of 24°C/h, and white eggs were the most tolerant developmental stage at the 12°C/h ramping heat rate, the development of an effective CA treatment that kills all white *T. leucotreta* eggs will control the further developed red and black head eggs as well. As was found for codling moth and oriental fruit moth eggs, *T. leucotreta* eggs also required a 2.0-h treatment under CA at 24°C/h ramping heat rate to achieve 100% mortality in the CAWB system.

Comparison of mortalities under RA treatment of the most thermotolerant instars of codling moth (fifth instar), oriental fruit moth (third and fourth instars) (Neven 2008) and *T. leucotreta* (third–fifth instars), suggested that, like codling moth, *T. leucotreta* was also more thermotolerant than oriental fruit moth. Neven (2008) also found that no instar of codling moth was more tolerant than another under 24°C/h CA treatments and that second–fourth instars of oriental fruit moth were equally tolerant to one another and more tolerant than first instars. In comparison with data from the current study, the most tolerant *T. leucotreta* instar (fourth) was more tolerant than both codling moth and oriental fruit moth larvae under the same conditions, with <60% mortality achieved at the 2.0-h time point when 100% mortality was achieved in the other two moth species. In contrast to findings from CAWB treatments on codling moth and oriental fruit moth (Neven 2008), in CATTS in-fruit tests, the fourth instars of both codling moth and oriental fruit moth were found to be the most tolerant to the treatment (Neven and Rehfield-Ray 2006b, Neven et al. 2006). However, CAWB treatments were conducted at the 24°C/h heat rate and CATTS treatments were tested at the 12°C/h heat rate. In the current study, fourth-instar *T. leucotreta* were the most tolerant stage at both the slow and fast ramping heat rates and seemed to be more tolerant of heated controlled atmosphere treatments than codling moth and oriental fruit moth. Fourth-instar *T. leucotreta* larvae may be more tolerant than fifth instars due to changing physiological aspects of fifth instars as it prepares to undergo pupation.

Effective CATTS treatments against codling moth and oriental fruit moth on peaches and nectarines required treatment times of 3 h at a ramping heat rate of 12°C/h and 2.5 h at 24°C/h (Neven et al. 2006). A variety of peach and nectarine cultivars evaluated for tolerance to these CATTS treatments showed similar posttreatment fruit quality at both heating rates, and the marketability was not adversely affected by the treatments (Obenland et al. 2005). CATTS treatments to control codling moth and oriental fruit moth in apples also has been confirmed using the 12°C/h heat rate (Neven and Rehfield-Ray 2006b), and some of the benefits of a CATTS treatment applied to pome fruit are firmer fruit with reduced storage scald (Neven et al. 2001). According to the results presented here, effective treatment against the most tolerant life stage of *T. leucotreta*, the fourth instar, would need to last for >2.5 h. At the slower heat rate, it would need to be substantially longer and would probably result in poorer fruit quality. Although *T. leucotreta* seemed to be more tolerant of the treatment than codling moth and oriental fruit moth, there is a possibility that the 24°C/h CA treatment can be developed into an effective treatment against *T. leucotreta.* Preliminary studies on the effect of heated CA treatments on two plum cultivars affected by *T. leucotreta* suggested that treatment at the 24°C/h ramping heat rate may help maintain storage quality of plums by reducing internal browning and maintaining firmer fruit (W. Witbooi, personal communication).

The use of combination treatments can possibly be taken a step further for citrus fruit, a major host of *T. leucotreta.* Currently, citrus exported from South Africa must undergo a lengthy cold treatment to control potential *T. leucotreta* larvae in the fruit. Combined heat treatments and cold storage provides improved control of codling moth (Neven 1994, Neven and Rehfield 1995). Unlike codling moth, *T. leucotreta* larvae cannot enter diapause and is consequently far more susceptible to low temperature treatments. Combining CATTS with cold storage to control *T. leucotreta* may reduce the severity of both treatments by reducing treatment times and as a result reduce adverse effects on fruit quality. A review and analysis of postharvest disinfestation treatments indicated that heat treatments are promising for citrus fruit (Pryke and Pringle 2008). High-temperature forced air treatments ranging from 45 to 48°C did not adversely affect the quality of navel orange and tangerine cultivars (Shellie et al. 1993, Sharp and McGuire 1996, Shellie and Mangan 1998). The analysis also indicated that

controlled atmosphere treatments have a high chance of success for citrus fruit.

The results obtained thus far merits further research into the use of heated modified controlled atmosphere treatments against *T. leucotreta.* The development of such treatments against several other target pest groups is reviewed in Heather and Hallman (2008) with the recommendation that heated modified controlled atmosphere treatments be further investigated and developed as they are promising alternative treatments.

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